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Bone Engineering of Maxillary Sinus Bone Deficiencies Using Enriched CD90+ Stem Cell Therapy: A Randomized Clinical Trial

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ABSTRACT

Bone engineering of localized craniofacial osseous defects or deficiencies by stem cell therapy offers strong prospects to improve treatment predictability for patient care. The aim of this phase 1/2 randomized, controlled clinical trial was to evaluate reconstruction of bone deficiencies of the maxillary sinus with transplantation of autologous cells enriched with CD90+ stem cells and CD14+ monocytes. Thirty human participants requiring bone augmentation of the maxillary sinus were enrolled. Patients presenting with 50% to 80% bone deficiencies of the maxillary sinus were randomized to receive either stem cells delivered onto a β-tricalcium phosphate scaffold or scaffold alone. Four months after treatment, clinical, radiographic, and histologic analyses were performed to evaluate de novo engineered bone. At the time of alveolar bone core harvest, oral implants were installed in the engineered bone and later functionally restored with dental tooth prostheses. Radiographic analyses showed no difference in the total bone volume gained between treatment groups; however, density of the engineered bone was higher in patients receiving stem cells. Bone core biopsies showed that stem cell therapy provided the greatest benefit in the most severe deficiencies, yielding better bone quality than control patients, as evidenced by higher bone volume fraction (BVF; 0.5 versus 0.4; $p = 0.04$). Assessment of the relation between degree of CD90+ stem cell enrichment and BVF showed that the higher the CD90 composition of transplanted cells, the greater the BVF of regenerated bone ($r = 0.56$; $p = 0.05$). Oral implants were placed and restored with functionally loaded dental restorations in all patients and no treatment-related adverse events were reported at the 1-year follow-up. These results provide evidence that cell-based therapy using enriched CD90+ stem cell populations is safe for maxillary sinus floor reconstruction and offers potential to accelerate and enhance tissue engineered bone quality in other craniofacial bone defects and deficiencies (Clinicaltrials.gov NCT00980278). © 2015 American Society for Bone and Mineral Research.

KEY WORDS: CLINICAL TRIALS; STEM CELLS; BIOENGINEERING; BONE μCT; DENTAL; IMPLANTS

Introduction

Oral and craniofacial bone defects secondary to congenital deformities, disease, and injury are very common and highly variable, representing a significant health care burden.[1] When these conditions are also associated with tooth loss or the congenital absence of teeth, alveolar bone of the jaw does not receive the functional stimulus innately produced by the teeth and their supporting structures and, as a result, further bone resorption results.[2] The consequences of these processes are severe horizontal and vertical bone deficiencies and inadequate bone volume to restore these areas of the jaw with functional and esthetic tooth replacements. In such cases, major alveolar bone reconstruction followed by dental prosthetic rehabilitation to reestablish form, function, and esthetics to these regions of the oral cavity are needed.[3]

When appropriate conditions are present, oral implant therapy serves as the most functional, esthetic, predictable, and therapeutic treatment option for the replacement of missing teeth.[4] However, a key determinant underlying the success of oral implant therapy is the qualitative and quantitative nature of the bone support into which implants are placed.[5] It has been long known that among those patients who seek oral implant therapy, a significant number lack

Received in original form November 24, 2014; revised form January 12, 2015; accepted January 20, 2015. Accepted manuscript online February 4, 2015.
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Journal of Bone and Mineral Research, Vol. 30, No. 7, July 2015, pp 1206–1216
DOI: 10.1002/jbmr.2464
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sufficient bone and require bone grafting procedures to enable implant placement and to improve function, predictability, and longevity of oral implants.[6]

Regardless of the extent or severity of the bone defects, the success of bone regenerative and reconstructive procedures used to correct them depends on the presence of osteogenic and vascular precursor cells resident in the surrounding tissues.[7] During the healing phase of regenerative therapies, some of these cells enter the defect and differentiate into osteoblasts that produce bone matrix and a vasculature that sustains architecture.[8] The dependence of these wound-healing and regenerative processes makes small, localized defects more manageable and predictable to treat, whereas larger reconstructions of severe deficiencies are much more challenging. Despite recent advances in tissue engineering and regenerative medicine, reconstruction of large defects still relies primarily on treatments involving large autogenous grafts, allografts, xenografts, and synthetic alloplastic materials.[9] Newer, more targeted cell and tissue-based therapies are needed to overcome the problematic limitations of traditional treatments.[10,11]

Stem cell therapy is a promising tissue engineering strategy to enhance tissue regeneration and promote de novo formation of both hard and soft tissues.[12–14] Recently, clinical reports have emerged that investigate cell therapy for craniofacial applications.[8,15–24] These early reports have had modest and mixed results, but a major limitation common to them is the limited characterization of the cell populations used for therapy. Insufficient knowledge regarding the cell population used as part of a stem cell therapy makes it difficult to understand the mechanisms underlying the study outcomes. The aims of this randomized, controlled, phase 1/2 clinical trial were to determine if autologous bone marrow–derived cells, including expanded CD90+ mesenchymal stem cells and CD14+ monocytes and macrophages, would be safe and efficacious in the treatment of large bone deficiencies of the edentulous maxilla. Bone deficiencies in these edentulous areas preclude restoration of the areas with oral implants and teeth because of the close proximity of the maxillary sinus. Surgical procedures involving augmentation of the sinus are routinely used to regenerate bone in these areas for oral implant installation, and many of these procedures involve the use of bone grafts.[25,26] As such, evaluation of regenerated and engineered bone in these maxillofacial sites enables better understanding of factors underlying successful treatment outcomes associated with cell therapy. The stem cell therapy employed in this study was compared with a control treatment using an alloplast device only.

Materials and Methods

Study design, patient selection, and randomization

Under an Investigational New Drug Application (US Food and Drug Administration IND# 13662), the FDA approved the treatment of up to 30 patients with the proposed stem cell therapy (Clinicaltrials.gov# NCT00980278). After US Food and Drug Administration and University of Michigan Institutional Review Board (IRB) approval, 30 patients with severe bone atrophy of the upper jaw and in need of bone reconstruction for oral implant and dental reconstruction were recruited to participate in this phase 1/2 randomized, controlled clinical trial. This sample size was chosen for feasibility rather than statistical precision. Through a computer-generated randomization schedule, half[15] of the patients were randomized to receive the stem cell therapy (ixymyelocel-t + beta-tricalcium phosphate [β-TCP] scaffold; Cerasorb, Curasan AG, Germany) and the other half randomized to receive the control treatment (β-TCP scaffold alone), with each subject only receiving one of the two possible treatments. Of the 30 enrolled, 4 withdrew from the study before undergoing any treatment-related procedures. Of the 26 participants, 2 dropped out before study completion (Fig. 1*A*). The primary outcome variables were bone volume fraction (BVF) and bone mineral density (BMD), and these were measured by histological and micro-computed tomography (μCT) analyses at 4 months post-treatment. Secondary outcome variables included: increase in linear radiographic bone height, increase in sinus bone volume, bone volume/initial graft volume ratio, soft tissue wound healing, postoperative pain score, clinical bone density on reentry of the grafted site, and quality-of-life assessment of treatment. Post hoc analyses included % CD90+ cells delivered relative to BVF and BMD.

Ixymyelocel-t production

In the study participants who were designated to be in the stem cell therapy group, 12 to 14 days before initial surgical treatment, 50 to 70 cc of bone marrow were aspirated from the posterior iliac crest. The cell processing for generation of the autologous cell product, ixmyelocel-t (Aastrom Biosciences Inc., Ann Arbor, MI, USA), has been previously described.[27] Briefly, the collected marrow was transferred to a sterile blood bag and bone marrow mononuclear cells (BMMNC) were purified by Ficoll density gradient centrifugation. BMMNC were then inoculated into a bioreactor, which is a proprietary computer-controlled, automated cell-processing unit, the Aastrom Replicell System (Aastrom Biosciences). This system incorporates single-pass perfusion in which fresh medium flows slowly over cells without retention of waste metabolites or differentiating cytokines. The culture medium consists of Iscove's modified Dulbecco's medium (IMDM), 10% fetal bovine serum, 10% horse serum, and 5 μM hydrocortisone. After cultivation for 12 days at 37 °C, 5% CO_2, with a ramped continuous medium perfusion schedule, the ixymyelocel-t product was harvested by trypsinization, washed in a physiologic buffer, and collected into a sterile bag, where it was stored until the time of transplantation. The final cell composition was composed of a mixture of bone marrow–derived cells, including different concentrations of expanded CD90+ mesenchymal stem cells, CD14+ monocytes/macrophages, and mononuclear cells from the original bone marrow aspirate.

Flow cytometric characterization of cell populations

All cell populations used in the stem cell therapies were characterized with respect to %CD90+ cells, %CD14+ cells, cell concentration of the final cell suspension, and number of cells delivered. In the flow cytometry analyses, all events were initially gated on light scatter (Forward Scatter versus Side Scatter) and then assessed for membrane integrity via 7AAD staining (ie, estimation of viability). Using the nucleic acid probe 7AAD, membrane-compromised events were excluded from further analysis. All subsequent plots were gated on scatter and 7AAD exclusion for phenotypic analysis. Thus, CD14+ and CD90+ measurements were based on gates for light scatter assessment/debris exclusion and "viability" (7AAD negative). As the original cell inoculum was cultured and the cells were expanded, there

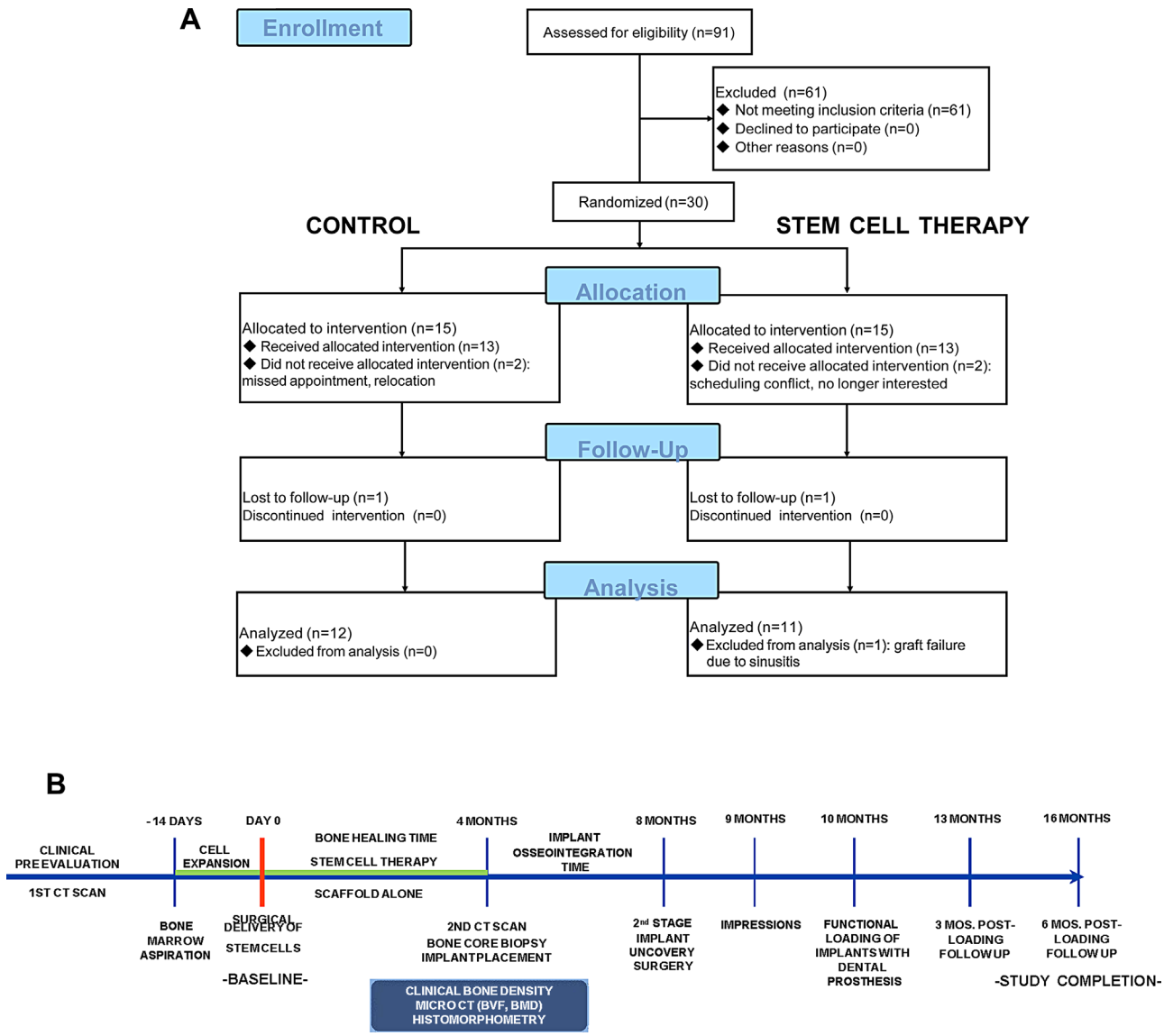

Fig. 1. Trial profile. (*A*) Consort diagram of patient distribution. (B) Study timeline.

was an increase in autofluorescence, which is displayed by the CD90+ and CD14+ Auto positive (CD14 + Auto +) populations. The CD14+ populations typically display varying levels of autofluorescence, which were separated by gates to distinguish the CD14+ autofluorescent-positive (CD14 + Auto +) and CD14+ autofluorescent-negative (CD14 + Auto-) populations.

Clinical procedures

Twelve to 14 days after the bone marrow aspiration, sinus elevation procedures were performed with simultaneous bone grafting according to the technique described by Tatum[28] (Supplemental Video S1). {Video S1} Briefly, a full-thickness mucoperiosteal crestal incision and flap was made with vertical extensions and the lateral aspect of the maxilla was exposed. A window osteotomy was then prepared on the lateral aspect of the maxilla to access the maxillary sinus cavity. The Schneiderian membrane of the sinus was then gently reflected from the floor of the sinus cavity and elevated 1 to 2 cm from its original height. The sinus cavity was then grafted under the elevated membrane by placing either the alloplast bone void material β-TCP alone or the β-TCP loaded with the cells. After placement of the graft, the sinus access window opening was then covered with a bioresorbable, occlusive collagen membrane, and the flap was sutured to attain primary closure. The bone graft material for these procedures serves to stimulate bone regeneration in the floor of the sinus cavity, resulting in vertical augmentation of the deficient bone height to enable future dental implant placement.

Bone height deficiencies ranged from 40% to 80%. Severe bone defects were classified as those where bone height deficiencies of >50% were present (ie, where the initial bone height was <50% of that required for oral implant placement with functional dental restoration). In the stem cell therapy group, the volume of cell suspensions delivered to each patient was determined by the volume of β-TCP used, and these

volumes were mixed at a 1:1 ratio 30 minutes before delivery. The final volume of β-TCP used in both control and stem cell therapy groups was determined by the extent of the bone height deficiency. The amount of scaffold placed at the time of grafting was recorded for each patient and correlated to the regenerated bone volume fraction (BVF = the proportion of regenerated bone composed of mineralized bone tissue) of the bone core biopsy. This measure was reported as "initial graft volume versus BVF." Additionally, the percentage of CD90+ cells within each of the patient cell populations was recorded and correlated with the regenerated BVF, and this measure reported as "% CD90+ cells versus BVF."

Clinical assessments, bone biopsy harvest, and oral implant installation

At 1, 2, and 4 weeks after surgery, the grafted sites were inspected and soft-tissue healing was evaluated with a wound-healing index (WHI) according to the following scheme:[29] 0 = mature wound healing; 1 = erythema; 2 = bleeding; 3 = flap mobility; 4 = suppuration; 5 = necrosis. Reentry procedures of the grafted sites were performed 4 months after sinus floor augmentation. Bone biopsy cores of approximately 2×10 mm in dimension were removed with a trephine drill (Ace Surgical Supply Co., Inc., Brockton, MA, USA) from areas corresponding to where the implants were going to be placed and the region(s) of the previous sinus graft. Upon drilling into the bone, clinical bone density was recorded according to tactile sensation as a measure of bone density. The bone biopsy cores were immediately fixed in 10% neutral-buffered formalin to enable histomorphometric and μCT analyses. Bone biopsies were scanned for μCT analyses and processed for histological analyses in the University of Michigan's School of Dentistry Histology Core. Oral implants (Straumann, Straumann AG, Basel, Switzerland) of 10 to 12 mm in length were placed in the grafted sites. Sinus augmentation procedures, and implant installations were performed by two different surgeons; bone core biopsy procedures were performed by one surgeon.

3D cone beam computed tomographic (CBCT) analysis

CBCT data model construction, registration, and visualization for volumetric assessment of the augmentation procedures was performed as previously described, with minor modifications.[30] Grayscale isotropic models were constructed from the CBCT images with a voxel dimension of $0.4 \times 0.4 \times 0.4$ mm. Three-dimensional surface models of the maxillary sinuses at baseline and post-treatment were constructed for each patient using ITK-SNAP (open-source software; http://www.itksnap.org). Baseline and post-treatment 3D models were registered according to anatomical landmarks, specifically the anterior nasal spine and the nasal crest of the maxillary bone within the floor of the nasal cavity. These regions were chosen because of the relative stability in the structure over a span of a few months. An automated, voxel-based registration method was performed with 3D Slicer (open-source software; http://www.slicer.org). Visualization and assessment of the regenerated bone were performed using CMF application software (developed at the M. E. Muller Institute for Surgical Technology and Biomechanics, University of Bern, Bern, Switzerland, under the funding of the Co-Me network; http://co-me.ch). Segmentation of the baseline and follow-up scans were performed using the ITK-SNAP software to detect the differences between the superimposed 3D images and to quantify the newly regenerated bone volume. The ratio of newly formed bone to amount of β-TCP used in the grafting procedure will be quantified and compared.

μCT analysis of bone biopsy cores

Nondecalcified bone cores were scanned and the data quantified using a 3D μCT 100 cabinet cone-beam μCT system (Scanco USA, Inc., Wayne, PA, USA). The specimens were fitted in a cylindrical sample holder, with the longitudinal axis of the bone core oriented in a horizontal position, and scanned at a resolution of $12 \times 12 \times 12$ μm. 3D isosurface images for the analysis of sample scans were constructed using the GEMS MicroView software (GE Healthcare, Little Chalfont, UK). Analysis was performed by a calibrated masked examiner as previously described with minor modifications.[31] The mean threshold grayscale values for bone and residual scaffold material (β-TCP) were used to calculate the BMD and the fractional bone volume (BV/TV) of newly formed mineralized bone at the graft site. The grafted site was demarcated visually as the region located superior (apical) to the dense, mature lamellar bone.

Bone histomorphometry

After scanning for μCT imaging, bone cores were processed for histological analysis. Histomorphometric analysis of decalcified, hematoxylin and eosin (H&E)-stained sections were performed to determine bone formation. Using an E-800 light microscope, histologic sections from each sample at each time point were scanned and imported into Adobe Photoshop (Adobe Systems, Inc., Mountain View, CA, USA). Identification of bone was based on morphology of stained tissue and the identification of cells lining (osteoblasts) and within (osteocytes) this tissue. Bone tissue area for each section was determined by dividing the total number of bone pixels by the total number of pixels in the tissue section based on color using Image Pro Plus Software.

Statistical analyses

Safety analyses were performed at each post-baseline visit and included reporting of adverse events by body system and by severity and relationship to the cell therapy, as assessed by the investigator. Statistical analysis was performed with the statistical software package R (R Foundation for Statistical Computing, Vienna, Austria). Plots represent means ± standard errors; differences in means between the two treatment groups were assessed with a two-sample *t* test. Correlation was based upon Pearson's product-moment correlation coefficient (r), with significance based on Fisher's Z-transformation. Statistical significance was defined as $p < 0.05$.

This trial is registered with ClinicalTrials.gov, number NCT00980278.

Results

Study design and patients

Throughout the course of the study, there were no serious, study-related adverse events that were reported in examination of comprehensive safety assessments during the trial (Supplemental Table S1). {TBL S1} Fig. 1*A* displays the Consort Diagram of the patient allocation to groups. After determination of study eligibility and enrollment, 4 patients elected not to pursue treatment as part of the study and a total of 26 patients were treated ($n = 13$ per group). The baseline demographic characteristics of all study participants are shown in Table 1,{TBL 1} and

Table 1. Patient Demographic Data

	Control	Stem cell therapy
No. of patients enrolled (no. of patients treated surgically)	15 (13)	15 (13)
Females	10	10
Mean age, years (range)	49.1 (26–65)	53 (27–66)
Ethnicity		
White	14	12
African American	1	1
Asian	0	2
Right maxilla/left maxilla	8/7	8/7
No. of patient withdrawals of consent before study entry	2	2
Mean baseline alveolar bone height (range)	5.0 (2.5–6.2)	3.5 (2.1–6.1)

there were no differences between the two treatment groups with regard to these parameters. Fig. 1*B* shows the trial sequence of events and timeline.

Complete surgical and prosthetic reconstruction of individual and multiple sites

When posterior teeth of the maxilla are removed, because of their proximity to the sinus cavities, the sinuses enlarge (pneumatize) and disuse atrophy of the bone in these regions poses significant constraints on the ability to place oral implants because of insufficient bone height, volume, and quality. For stable placement and restoration of oral implants in the posterior maxilla in these cases, it is desirable to have a minimum of 10 mm of bone height between the alveolar crest and the maxillary sinus cavity.[32,33] All patients treated in this study were in need of bone-regenerative procedures for oral implant placement because of bone height deficiencies ranging from 50% to 80%. Additionally, all patients treated were in need of either localized site bone reconstruction (for replacement of one tooth) or multiple site bone reconstruction (for replacement of up to four teeth) in this area. The clinical sequence of treatment procedures for reconstruction of localized bone deficiencies for single-tooth replacements in the control and stem cell therapy groups involved grafting with the β-TCP scaffold or the stem cells on the scaffold as part of a routine sinus lift procedure. The clinical procedures were no different between treatment groups, and in both groups, favorable function and esthetics were achieved with the final tooth restorations (Supplemental Fig. S1). The clinical sequence of treatment procedures for reconstruction of larger regions of bone deficiencies for areas requiring the replacement of multiple teeth was slightly variable, depending on the number of sites treated and respective number of teeth being replaced (between two and four) (Fig. 2).

The clinical surgical parameters of the two treatments were equivalent between groups (Supplemental Table S2). There was one graft failure in the treatment group and one implant failure in the control group. In addition to the clinical surgical treatment parameters documented, the soft-tissue wound healing after grafting was evaluated. One, two, and four weeks postoperatively after initial regenerative treatment, there was no difference in the soft-tissue profiles of healing between treatment groups, nor was there a difference in postoperative discomfort between groups (Supplemental Fig. S2).

Because of the high morbidity and postoperative discomfort associated with large oral and craniofacial reconstructive procedures, one final clinical assessment at the conclusion of treatment involved a survey of patient psychosocial factors associated with the stem cell therapy treatment. In this survey of patient quality-of-life variables (Supplemental Fig. S3), despite undergoing the bone marrow aspiration procedure for isolation of the autologous cells, patients in the stem cell therapy group were equally satisfied with their treatment compared with those undergoing standard of care procedures, and all indicated they would have these procedures performed again if needed. One of the patients in the cell therapy group indicated that the marrow-harvesting procedures resulted in significant discomfort, yet this individual also indicated that he/she would have the procedure performed again if needed. One patient in the control group indicated that he/she would not have the procedure performed again if ever necessary.

Linear radiographic bone height changes

Radiographic bone height is a key clinical determinant to assess the need for bone grafting in the areas of the posterior maxilla in proximity to the maxillary sinuses. Hence, linear radiographic changes in bone height before and after bone graft reconstruction were evaluated. Significant changes in bone height were achieved in the control and stem cell therapy groups (Fig. 3*A–D, G–J*; Table 2). {FIG3}{TBL 2} Alveolar bone height of the posterior maxilla beneath the maxillary sinus increased two- and threefold in most cases and up to fivefold in two of the cases in the stem cell therapy group. Overall, there was no difference in the mean linear radiographic bone height changes between the treatment groups (Table 2). After oral implant placement and 6 months of functional loading of implants with dental restorations, the bone consolidation around the implants remained stable radiographically in both groups for all patients (Fig. 3*E, F, K, L*).

Correlation of CD90+ cells with BVF

CD90+ mesencyhmal stem cells have been previously shown to have strong regenerative and bone differentiation potential. The stem cell therapy described utilized autologous cell populations, which, relative to the initial bone marrow aspirate, are enriched 100-fold for CD90+ cells and CD14+ cells during the cell expansion process (Fig. 4*A*). Between patients receiving the stem cell therapy, there was heterogeneity in the final percentage of enriched CD90+ cells ranging from 15% to 40% (Supplemental Table S3). β-TCP particles were used as a cell scaffold to deliver the cells into the bone defect, and 4 months after delivery of the cells or the scaffold alone, bone biopsies from the regenerated tissue were retrieved and evaluated with 3D μCT. Intact bone

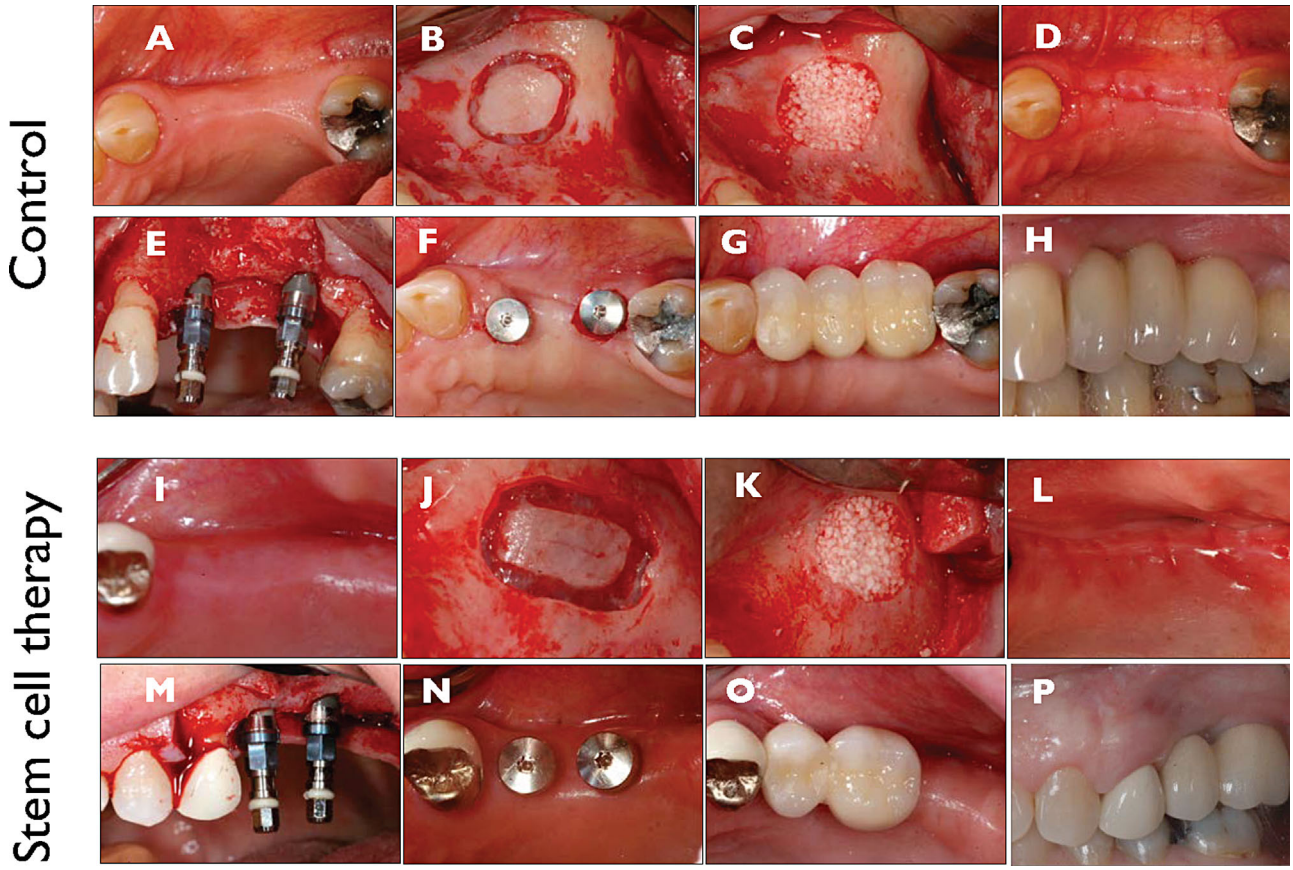

Fig. 2. Clinical treatment sequence for multiple site sinus bone graft reconstruction of severe bone atrophy. (*A*) Clinical images of the occlusal (top) view of the initial edentulous region of patients treated in the (*A*) control or (*I*) stem cell therapy group. Buccal (lateral) views of the surgical site located above the edentulous region in the (*B*) control and (*J*) stem cell therapy groups show preparation of a window osteotomy in the lateral aspect of the maxilla for access to the Schneiderian membrane of the maxillary sinus cavity. After elevation of the maxillary sinus, the β-TCP (*C*) scaffold or stem cells (*K*) on the scaffold are placed in the sinus cavity and, after closure of the surgical area, occlusal views of the edentulous areas 2 weeks after healing are shown (*D, L*). Four months after control or stem cell treatment, oral implants (*E, M*) are placed in the edentulous areas of the grafted regions and allowed to integrate into the bone for 6 months (*F, N*). At 6 months, implants in both treatment groups are biomechanically loaded with functional dental prosthetics, restoring the edentulous areas with teeth, as shown in the occlusal (*G, O*) and buccal views (*H, P*).

cores could not be harvested from 3 patients because of technical challenges in the retrieval of these specimens, yet these analyses enabled the determination of the extent to which regenerated tissue was composed of residual β-TCP graft particles and regenerated bone tissue (Fig. 4*B*). In evaluating all the bone cores retrieved, there was a significant negative correlation ($r = -0.75$, $p = 0.02$) between the amount of graft placed and the proportion of bone formed (BVF) within the regenerated tissue (Fig. 4*C*). Thus, depending on the size of the defect, as the amount of scaffold material increased, the quality of the regenerated bone tissue decreased (as measured by BVF). However, it was striking to note that in the stem cell therapy group, the percentage of CD90+ cells in different cell populations yielded an enhancement in the regenerated bone quality, with it being a significant positive correlation between percent CD90+ cells and BVF ($r = 0.56$; $p = 0.05$) (Fig. 4*C*). This relationship was consistent with the clinical evaluation of the regenerated bone density, where the clinical bone density was highest in the patients treated with the stem cell therapy (Supplemental Fig. S4).

3D volumetric changes in bone formation

CBCT was used to evaluate 3D changes in the bone volume within the treated areas of the sinus cavity. Overall, there was a significant increase in bone volume in both treatment groups, but no difference between groups in the ratio of regenerated bone volume to initial grafted volume was observed (Fig. 5*A, B*; Table 2). Using μCT and histological analyses to evaluate the qualitative nature of the de novo bone, it appeared that the BVF of the regenerated bone was higher in biopsies retrieved from patients who received the stem cell therapy. Quantitatively, the BVF for biopsies from the stem cell therapy group (0.49) was higher than that for the control group (0.43) (Table 2). Additionally, the bone quality of regenerated bone was significantly enhanced in patients receiving the stem cell therapy in the most severe deficiencies (>50% deficiency in bone height). In these cases, the regenerated bone biopsies from patients receiving the stem cell therapy had a significantly greater BVF than those cores harvested from the control group (0.5 versus 0.4, respectively; $p = 0.04$) (Fig. 5*C*).

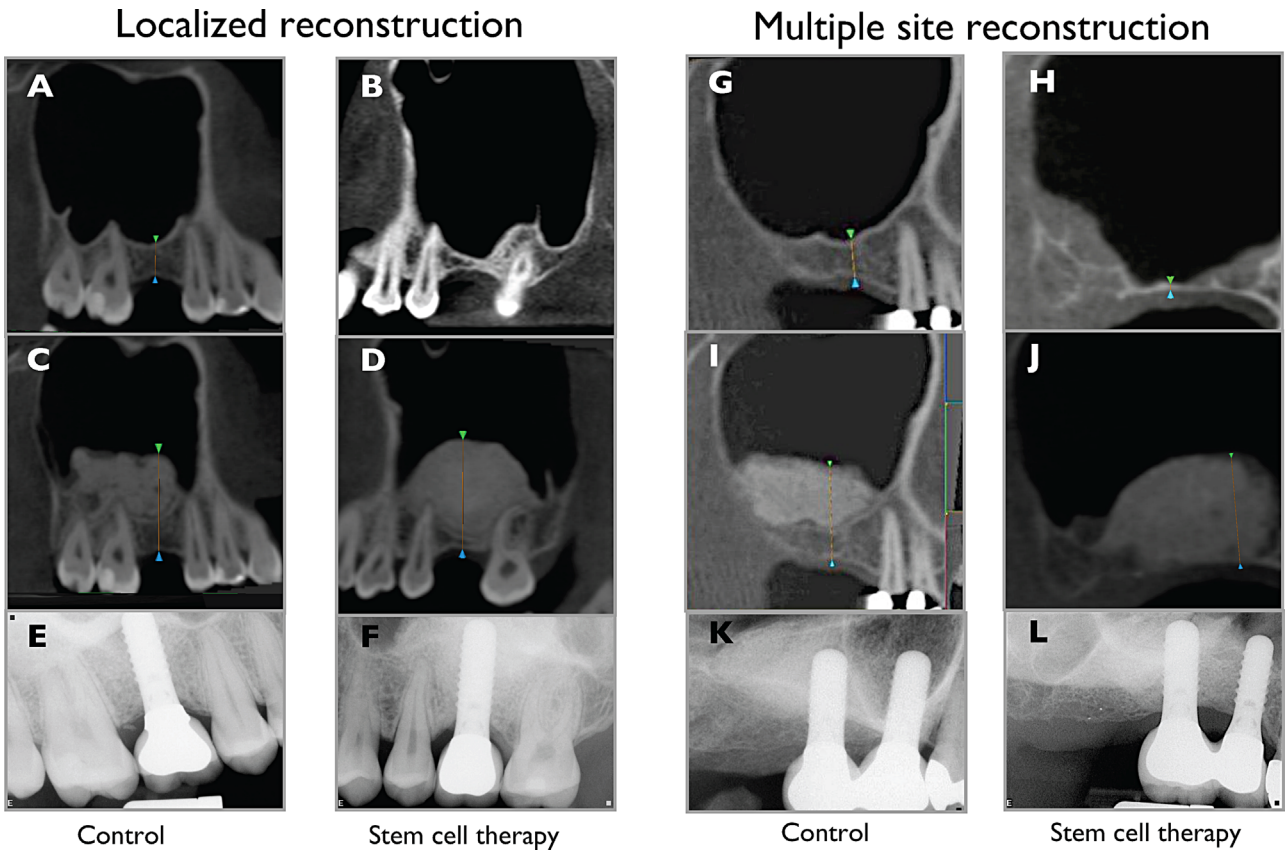

Fig. 3. Radiographic evaluation of sinus grafts and implant stability 6 months after functional loading. Cross-sectional images from CBCT scans showing initial bone height in localized reconstructions (single site/tooth) of the (*A*) control and (*B*) stem cell therapy groups and bone height 4 months after treatment (*C, D*) in both groups. (*E, F*) Periapical radiographs show bone consolidation around the implants 6 months after functional restoration of the restored areas with a tooth. Cross-sectional images from CBCT scans showing initial bone height in multiple site reconstructions (2 to 4 teeth) of the control (*G*) and stem cell therapy (*H*) groups and bone height 4 months after treatment (*I, J*) in both groups. (*K, L*) Periapical radiographs show bone consolidation around the implants 6 months after functional restoration of the restored areas with teeth.

Discussion

The prospect that stem cell therapies offer significant advantages over traditional approaches for oral and craniofacial reconstruction has led to the development of an immense body of work characterizing different stem cell populations and their regenerative potential. Nonetheless, to date, there has been limited translation of this work toward craniofacial applications.[21–25] In this report, we describe an autologous stem cell therapy used to engineer bone tissue in moderate-severe bone deficiencies of the maxilla in close proximity to the maxillary sinuses. De novo bone regeneration was sufficient to stably place oral implants, which were ultimately used to functionally support dental prostheses. A key finding from this study was that compared with the control group, better-quality bone was formed in patients who received the stem cell therapy and bone quality significantly correlated with the percentage of autologous CD90+ cells transplanted. Additionally, patients who underwent the cell therapy indicated that they were completely satisfied with the treatment outcome, did not have to significantly alter daily life activities as a result of treatment, and would have the same procedures performed again if needed.

Autogenous, allogeneic, and alloplast bone "void fillers" are typically grafted in the sinus cavity, and all modalities have been shown to generate sufficient height and bone volume for stable

Table 2. Cone Beam and μCT 1° and 2° Outcome Measures

Outcome variables	Control	Stem cell therapy
Increase in linear radiographic bone height (mean, mm ± SD)	12.8 (2.8)	12.2 (3.3)
Increase in sinus bone volume (mean, cm^3 ± SD)	2.1 (0.9)	1.8 (1.0)
Final bone volume/initial graft volume ratio (mean, ± SD)	0.64 (0.2)	0.51 (0.3)
Bone volume fraction of bone core (μCT) (mean, ± SD)	0.43 (8.1)	0.49 (7.2)
Bone mineral density of bone core (μCT) (mean, mg/mm^3 ± SD)	0.79 (0.05)	0.78 (0.02)

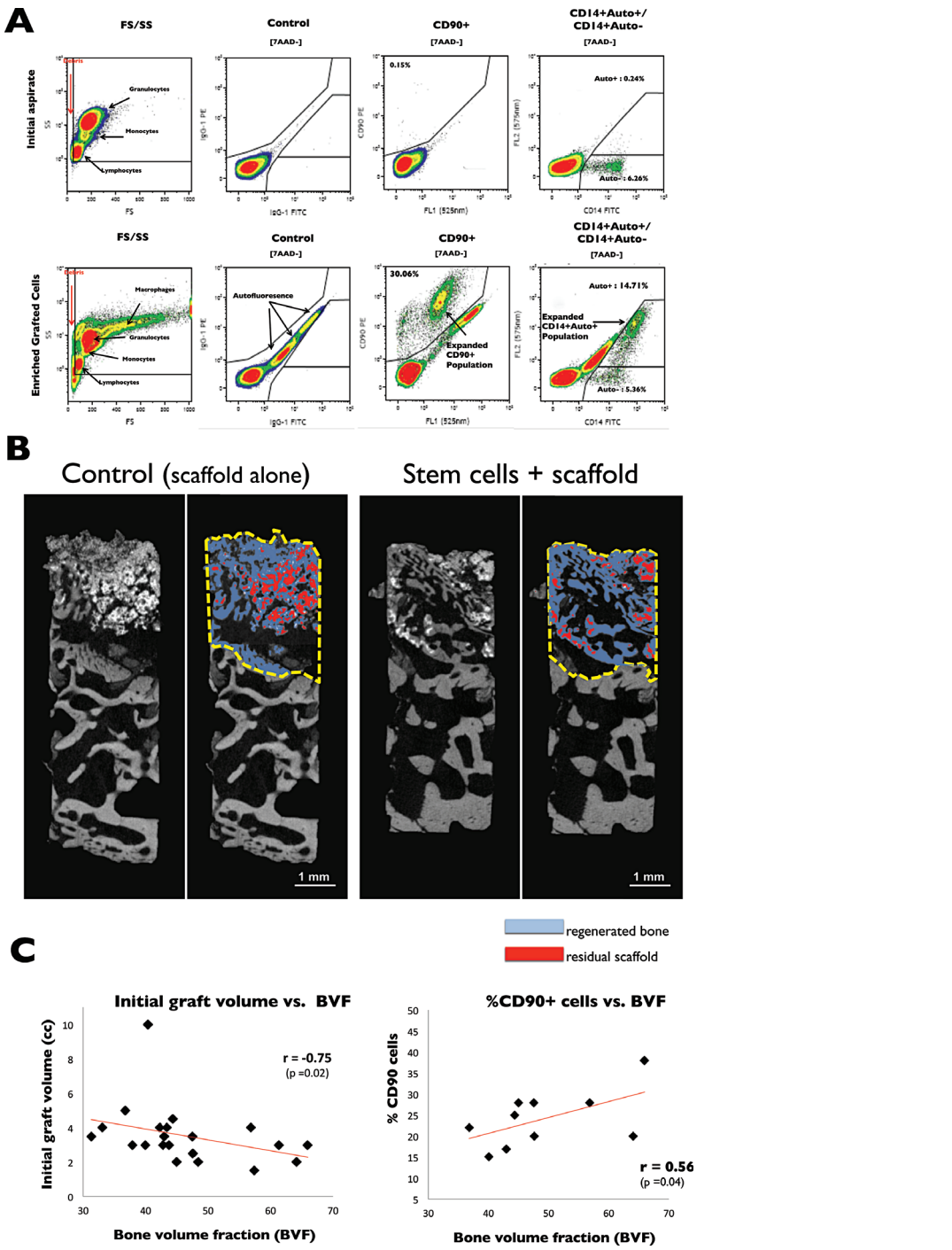

Fig. 4. Cell populations with higher percentages of CD90+ cells yield better quality of regenerated bone. (*A*) Representative flow cytometric analysis from a patient shows enrichment of CD90+ and CD14+ cell populations from the initial aspirate to the final cell population used for treatment. All events are initially gated on light scatter (FS versus SS) and then assessed for membrane integrity via 7AAD staining (ie, estimation of viability). An increase in autofluorescence throughout the cell expansion process can be found in the Control, CD90 +, and CD14 + Auto +/CD14 + Auto- plots. The CD14+ populations display varying levels of autofluorescence, which are separated by gates to distinguish the CD14+ autofluorescent-positive (CD14 + Auto +) and CD14+ autofluorescent-negative (CD14 + Auto-) populations. (*B*) μCT images of representative bone biopsies from the control (scaffold only) and stem cell therapy groups clearly show residual grafted scaffold (β-TCP) particles in the grafted zone 4 months after grafting. The zone of regenerated bone is delineated from the native bone (yellow hashed line) and in the stem cell group is composed of more bone relative to residual graft compared with the control group. (*C*) For all patients between both the control and stem cell therapy groups, there was a significant inverse relationship between the amount of scaffold placed at the time of grafting and the bone volume fraction (the proportion of regenerated bone composed of mineralized bone tissue) of the bone biopsy (Initial graft volume vs. BVF). For only those patients receiving the stem cell therapy, there was a significant positive correlation between the percentage of the CD90+ cells (within the cell populations delivered) and the BVF (% CD90+ cells vs. BVF).

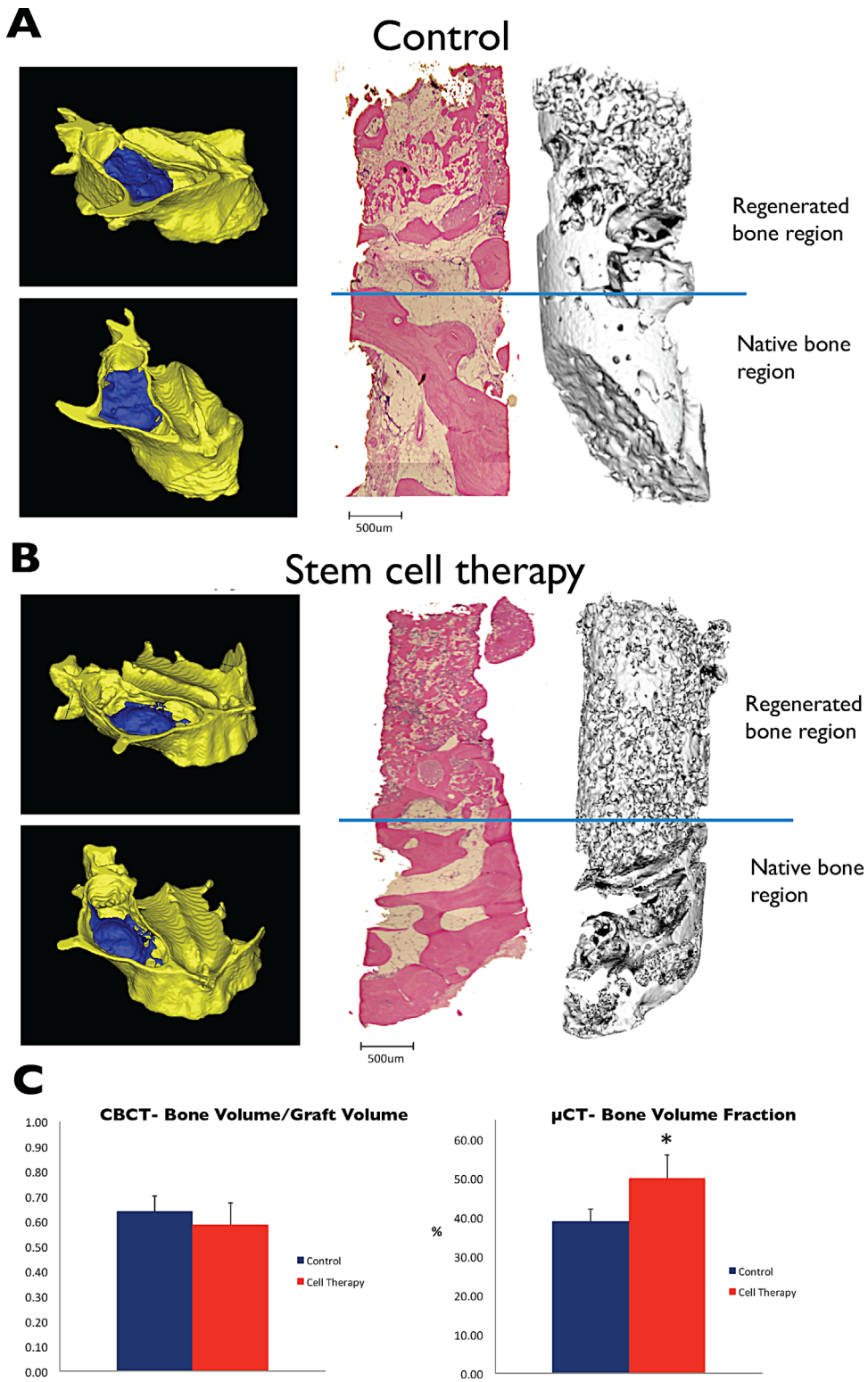

Fig. 5. Better bone quality with stem cell therapy in treating severe defects (>50% bone height deficiency). Representative images of 3D reconstructions of occlusal and lateral open views into the maxillary sinus cavity of the skull show the bone volume that was grafted (blue) in the control (*A*) and stem cell therapy (*B*) groups in severe bone defects. Histological and corresponding µCT images of bone biopsies harvested from the grafted regions of the two groups show a greater degree of mineralized bone tissue in the stem cell therapy group. (*C*) CBCT analysis of the bone volume/graft volume ratio (Bone Volume/Graft Volume) was no different between the control and stem cell therapy groups in treating severe defects; µCT analyses of the bone biopsies revealed that compared with the control, BVF was significantly higher in the stem cell therapy group in treating severe defects.

placement of oral implants to support functional tooth replacements.[26] Additionally, both the Schneiderian membrane of the sinus and the osseous sinus cavity have innate bone-regenerative potential without bone grafts or substitutes.[25] Nonetheless, it is widely accepted that autogenous grafts produce the most viable and highest-quality bone (ie, most highly mineralized, most cellular, most vascularized), whereas alloplastic grafts, being osteoconductive, yield regenerated bone tissue composed of high percentages of the nonresorbed alloplast long after initial turnover and remodeling occurs. It is the bone quality that ultimately is the greatest predictor of long-term success of oral implant therapy. In our study, both control and stem cell therapies yielded sufficient bone height and volume for the placement of oral implants, yet a key finding was that regenerated bone in the stem cell group was of higher quality, defined by the BVF. This was particularly apparent in the larger reconstructions that treated the most severe deficiencies. In these cases, the bone tissue formed in the control group was composed of a higher proportion of residual alloplast β-TCP carrier, whereas the stem cell therapy yielded a greater proportion of regenerated bone being composed of viable, highly vascular, mineralized bone tissue.

Sauerbier and colleagues reported on the use of concentrated mononuclear cells from bone marrow aspirates for sinus floor reconstruction where cells were not expanded but, instead, separated from the aspirate at the time of grafting.[34] It was demonstrated histologically that 10% to 40% of the newly formed bone tissue was mineralized 3 to 4 months after grafting. There was no characterization of the implanted cells and thus the cell phenotype of the cell populations used was not determined. In our study, the range of the BVF of regenerated bone in the control group was in alignment with the results from this report, being from 31% to 57% in the control group. The BVF range was higher in the stem cell therapy group, being from 36% to 65%.

De novo bone formed in sinus reconstructive procedures is primarily dependent upon osteogenic and vascular cells from the sinus cavity to infiltrate and remodel the graft material to ultimately form bone. In our study, the provision of a cellularized graft to the defect appeared to accelerate the process of remodeling, demonstrated by less residual graft particles being present 4 months after treatment in biopsy samples obtained in the experimental group. This concept of accelerated remodeling is supported by the findings reported in one of our recent clinical studies in which bone healing was accelerated with cell therapy in small, localized tooth-extraction defects.[35] The approach of using a cellularized graft for sinus grafting procedures was also evaluated by Gonshor and colleagues.[36] They used a commercially available allogeneic cellular graft that contained approximately 50,000 CD105+ cells/cc. The primary outcomes evaluated in the study were radiographic changes in bone height and vital bone content of bone biopsies. Though there was an enhancement in the vital bone content of the cellularized allograft versus conventional allograft alone, the average vital bone content in the cellularized graft was 32%, which was still slightly lower than the BVF of the control group in our study. In our study, cell concentrations ranged from 5 to 15 million cells/cc with a range of 15 to 80 million total cells being transplanted, depending on the severity of the initial deficiency. Comparative analysis of treatment outcomes of other regenerative modalities for sinus augmentation is included in Supplemental Table S4.{TBL S4}

It is well established that the bone marrow contains mixed populations of stem and progenitor cells, which have regenerative potential and potent trophic properties in their ability to affect cells resident within the recipient site. Our study used highly characterized cell populations enriched up to 100-fold in mesenchymal CD90+ cells through the cell expansion process, and each cell population is characterized independently, providing important information about the phenotype of the cells from each patient. This is of particular importance because specific cell characteristics seem to influence regenerative outcomes. To our knowledge, our study is the first report to evaluate how cell phenotype correlates to clinical regenerative outcomes, showing that irrespective of the number of cells delivered, the best bone quality was achieved in patients whose cell populations had the highest percentages of CD90+ cells. This specific relationship needs to be further evaluated in a larger number of patients, but this finding could be highly important in optimizing and personalizing clinical cell therapy protocols to meet specific needs of different patients.

Like most regenerative studies applying novel therapies, our clinical trial was designed to evaluate safety and efficacy; however, unlike most studies, we also aimed to acquire information relative to the treatment protocol from the patient perspective. This quality-of-life assessment is often overlooked or not reported when trying to determine the initial feasibility of emerging therapies; yet, if the therapy is deemed effective, these factors could underscore the acceptance and widespread use of these procedures. Traditional treatments for large oral and craniofacial defects routinely utilize large autogenous grafts, which often require significant recovery time and because of the associated postoperative pain and distress, most patients would not elect to undergo them again if necessary.[37] In contrast, our study found that at the completion of treatment in patients receiving the cell therapy, all participants reported that the treatment regimen and procedures involved did not significantly impact their daily life activities and that, if necessary, they would undergo them again.

To conclude, this randomized, controlled trial is the first of its kind to evaluate a stem cell therapy for craniofacial bone regeneration in the severely atrophic maxilla. It was demonstrated that stem cell therapy yielded better regenerated bone quality than in the control group and, further, that this enhancement correlated significantly with the percentage of CD90+ stem cells within the cell population grafted. This study provides evidence that stem cell therapy could be considered for treatment of other challenging oral and craniofacial bone defects (ie, segmental/continuous defects) or combined with other treatment modalities where accelerated bone healing and highly viable bone is desired. Other important considerations for continual development and optimization of this approach include: the source of cells, use of animal sera substitutes (ie, autologous serum), the cell expansion protocol, and the scaffold material for cell delivery.

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

Funding was provided by ITI Foundation (WVG), the Burroughs Wellcome Fund (CAMS; DK), National Institutes of Health (NIH/NCRR UL1RR024986; WVG) and Straumann Inc. for their generous donation of dental implants and component parts.

The authors thank Dr Andrew Eisenberg for performing the bone marrow aspirations and also thank Ronnda Bartel, Judy

Douville, Anna Galloro, Christina Huffman, Mary Layher, Tina Lucas, and Aastrom Biosciences, Inc. for administrative, clinical, and laboratory support.

Authors' roles: Study design: DK and WG. Study conduct: DK, GA, ST, MP, and WG. Data collection: DK, GA, ST, ST, AT, MP, IR, FW, and AL. Data analysis: DK, AT, and WG. Data interpretation: DK, GA, ST, MP, and WG. Drafting manuscript: DK and WG. Revising manuscript content: DK, GA, ST, and WG. Approving final version of manuscript: DK, GA, ST, AT, MP, IR, FW, AL, and WG. DK and WG take responsibility for the integrity of the data analysis.

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